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Published
With international search report.

(54) Title: ANIMAL FEED ADDITIVES COMPRISING XYLANASE

(57) Abstract

The present invention relates to animal feed additives, which additives comprise a monocomponent xylanase derived from a strain of *Humicola*, a strain of *Thermoascus*, a strain of *Chaetomium*, a strain of *Mucor*, a strain of *Talaromyces*, a strain of *Malbranchea*, a strain of *Myceliophthora*, a strain of *Thielavia*, a strain of *Byssochlamus*, or a strain of *Paecilomyces*. In other aspects, the invention relates to monocomponent xylanase preparations, DNA constructs, recombinant expression vectors, host cells, and methods of producing monocomponent xylanase preparations.

CLAIMS

1. An animal feed additive, which additive comprises a monocomponent xylanase derived from a strain of *Humicola*, a strain of *Thermoascus*, a strain of *Chaetomium*, a strain of *Mucor*, a strain of *Talaromyces*, a strain of *Malbranchea*, a strain of *Myceliophthora*, a strain of *Thielavia*, a strain of *Byssochlamus*, or a strain of *Paecilomyces*.

2. The animal feed additive according to claim 1, in which the monocomponent xylanase is derived from a strain of *Thermomyces*.

3. The animal feed additive according to claim 2, in which the monocomponent xylanase is derived from a strain of *Thermomyces lanuginosus*.

4. The feed additive according to claim 3, in which the monocomponent xylanase is derived from the strain *Thermomyces lanuginosus*, DSM 4109, or a mutant or a variant thereof.

5. The feed additive according to any of claims 1-4, in which the monocomponent xylanase has immunochemical properties identical or partially identical to those of a purified xylanase, which is either

a) derived from the strain *Thermomyces lanuginosus*, DSM 4109; or

b) encoded by the DNA sequence presented as SEQ ID NO: 1; or

c) encoded by the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133.

6. The feed additive according to any of claims 1-5, in which the monocomponent xylanase is derived from a host cell carrying a gene encoding the xylanase component.

7. The feed additiv according to any of claims 1-6, in which the monocomponent xylanas is

a) encoded by the DNA sequence presented as SEQ ID NO: 1, or by the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

b) encoded by a DNA sequence analogue to the xylanase encoding part of the DNA sequence presented as SEQ ID NO: 1, or to the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133, which analog DNA sequence either

i) is homologous to the xylanase encoding part of the DNA sequence presented as SEQ ID NO: 1, or to the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

ii) hybridizes with the same oligonucleotide probe as the xylanase encoding part of the DNA sequence presented as SEQ ID NO: 1, or with the DNA sequence obtainable from th plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

iii) encodes a polypeptide which is at least 70% homologous to the polypeptide encoded by the DNA sequence presented as SEQ ID NO: 1, or to the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

iv) encodes a polypeptide which is immunologically reactive with an antibody raised against the purified xylanase derived from the strain *Thermomyces lanuginosus*, DSM 4109, or encoded by the DNA sequence presented as SEQ ID NO: 1, or the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133.

8. The feed additive according to any of claims 1-7, in which the mono-component xylanase is further characterized by having a residual enzyme activity of more than 96% after incubation for 60 minutes at pH 6.0 and 60°C.

9. The feed additive according to any of claims 1-7, in which the monocomponent xylanase is further characterized by having a residual enzyme activity of more than 83% after incubation for 60 minutes at pH 6.0 and 65°C.

10. The feed additive according to any of claims 1-7, in which the monocomponent xylanase is further characterized by having a residual enzyme activity of more than 20% after incubation for 60 minutes at pH 6.0 and 70°C.

11. The feed additive according to any of claims 1-7, in which the monocomponent xylanase is further characterized by having a residual enzyme activity of more than 10% after incubation for 60 minutes at pH 6.0 and 75°C.

12. The feed additive according to any of claims 1-11, which comprises one or more additional feed enhancing enzymes.

13. The feed additive according to claim 12, which comprises one or more additional feed enhancing enzymes selected from the group consisting of an α-galactosidase, a β-galactosidase, a phytase, a galactanase, a xylanase, and a protease.

14. The feed additive according to any of claims 1-13, provided in the form of a dry composition, preferably a coated or uncoated granulate, or provided in the form of a stabilized liquid composition, preferably an aqueous or oil-based composition.

15. A monocomponent xylanase preparation, in which preparation the xylanase component is derived from a strain of *Humicola*, a strain of *Thermoascus*, a strain of *Chaetomium*, a strain of *Mucor*, a strain of *Talaromyces*, a strain of *Malbranchea*, a strain of *Myceliophthora*, a strain of *Thielavia*, a strain of *Byssochlamus*, or a strain of *Paecilomyces*.

16. The monocomponent xylanas preparation according to claim 15, in which preparation the xylanase component is derived from a strain of *Thermomyces*.

17. The monocomponent xylanase preparation according to claim 16, in which preparation the xylanase component is derived from a strain of *Thermomyces lanuginosus*.

18. The monocomponent xylanase preparation according to claim 17, in which preparation the xylanase component is derived from the strain *Thermomyces lanuginosus*, DSM 4109, or a mutant or a variant thereof.

19. The monocomponent xylanase preparation according to any of claims 15-18, in which the xylanase component has immunochemical properties identical or partially identical to those of a purified xylanase which is either

a) derived from the strain *Thermomyces lanuginosus*, DSM 4109; or

b) encoded by the DNA sequence presented as SEQ ID NO: 1; or

c) encoded by the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133.

20. The monocomponent xylanase preparation according to any of claims 15-19, in which the xylanase component is derived from a host cell carrying a gene encoding the xylanase component.

21. The monocomponent preparation according to claim 22, in which the xylanase component is

a) encoded by the DNA sequence presented as SEQ ID NO: 1, or by the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

b) encoded by a DNA sequence analogue to the xylanase encoding part of the DNA sequence presented as SEQ ID NO: 1, or to the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133, which analog DNA sequence either

i) is homologous to the xylanase ncoding part of the DNA sequence presented as SEQ ID NO: 1, or to the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

ii) hybridizes with the same oligonucleotide probe as the xylanase encoding part of the DNA sequence presented as SEQ ID NO: 1, or with the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

iii) encodes a polypeptide which is at least 70% homologous to the polypeptide encoded by the DNA sequence presented as SEQ ID NO: 1, or to the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

iv) encodes a polypeptide which is immunologically reactive with an antibody raised against the purified xylanase derived from the strain *Thermomyces lanuginosus*, DSM 4109, or encoded by the DNA sequence presented as SEQ ID NO: 1, or the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133.

22. The monocomponent xylanase preparation according to any of claims 15-21, in which the xylanase component has a residual enzyme activity of more than 96% after incubation for 60 minutes at pH 6.0 and 60°C.

23. The monocomponent xylanase preparation according to any of claims 15-21, in which the xylanase component has a residual enzyme activity of more than 83% after incubation for 60 minutes at pH 6.0 and 65°C.

24. The monocomponent xylanase preparation according to any of claims 15-21, in which the xylanase compon nt has a residual enzyme activity of more than 20% after incubation for 60 minutes at pH 6.0 and 70°C.

25. Th monocomponent xylanase pr paration according to any of claims 15-21, in which the xylanase component has a residual enzyme activity of more than 10% after incubation for 60 minutes at pH 6.0 and 75°C.

26. A DNA construct comprising a DNA sequence encoding a xylanase component, which DNA sequence comprises:

a) the xylanase encoding part of the DNA sequence presented as SEQ ID NO: 1, or the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

b) a DNA sequence analogue to the xylanase encoding part of the DNA sequence presented as SEQ ID NO: 1, or to the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133, which analog DNA sequence either

i) is homologous to the xylanase encoding part of the DNA sequence presented as SEQ ID NO: 1, or to the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

ii) hybridizes with the same oligonucleotide probe as the xylanase encoding part of the DNA sequence presented as SEQ ID NO: 1, or with the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

iii) encodes a polypeptide which is at least 70% homologous to the polypeptide encoded by the DNA sequence presented as SEQ ID NO: 1, or to the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133; or

iv) encodes a polypeptide which is immunologically reactiv with an antibody raised against the purified xylanase deriv d from the strain *Thermomyc s lanuginosus*, DSM 4109, or ncoded by the DNA sequence presented as SEQ ID NO: 1,

or the DNA sequence obtainable from the plasmid in the strain *Saccharomyces cerevisiae* DSM 10133.

27. The DNA construct according to claim 26, in which the DNA sequence encoding the xylanase component is derived from a strain of *Humicola*, a strain of *Thermoascus*, a strain of *Chaetomium*, a strain of *Mucor*, a strain of *Talaromyces*, a strain of *Malbranchea*, a strain of *Myceliophthora*, a strain of *Thielavia*, a strain of *Byssochlamus*, or a strain of *Paecilomyces*.

28. The DNA construct according to claim 27, in which the DNA sequence encoding the xylanase component is derived from a strain of *Thermomyces*.

29. The DNA construct according to claim 28, in which the DNA sequence encoding the xylanase component is derived from a strain of *Thermomyces lanuginosus*.

30. The DNA construct according to claim 29, in which the DNA sequence is derived from, or produced on the basis of, a DNA library of *Thermomyces lanuginosus*, DSM 4109, or a mutant or a variant thereof.

31. A recombinant expression vector comprising a DNA construct according to any of claims 26-30.

32. A host cell comprising a DNA construct according to any of claims 26-30, or a recombinant expression vector according to claim 31.

33. The host cell according to claim 32, which is a eukaryotic cell, in particular a fungal cell, preferably a yeast cell or a filamentous fungal cell.

34. The host cell according to claim 32, which cell belongs to a strain of *Aspergillus*, in particular a strain of *Aspergillus niger* or *Aspergillus oryzae*.

35. A method of producing a monocomponent xylanase preparation according to claims 15-25, which method comprises culturing the host cell according to any of claims 32-34 under conditions permitting the production of the xylanase component, followed by recovery of the xylanase component from the culture.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00046

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 9/24, C12N 9/42, A23K 1/165
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIL, CLAIMS, JAPIO, MEDLINE, BIOSIS, EMBASE, CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9104673 A1 (NOVO NORDISK A/S), 18 April 1991 (18.04.91), see abstract, examples and claims	1-14
A	--	15-35
X	APPL MICROBIOL BIOTECHNOL, Volume 39, 1993, J. Gomes et al, "Production of a high level of cellulase-free xylanase by the thermophilic fungus Thermomyces lanuginosus in laboratory and pilot scales using lignocellulosic materials", page 700 - page 707, see abstract, see page 703 paragraph 4 and page 704 fig 3	15-35
A	--	1-14

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 April 1996	30 -04- 1996
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl-Olof Gustafsson Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00046

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOTECHNOLOGY LETTERS, Volume 15, No 4, April 1993, Lischnig T et al, "Thermostability of endo-beta-xylanase from the thermophilic fungus thermomyces lanuginosus", page 411 - page 414, see summary page 411 and fig 1 page 412	15-35
A	--	1-14
X	ENZYME MICROB. TECHNOL., Volume 16, April 1994, Mustafa Alam et al, "Production and characterization of thermostable xylanases by *Thermomyces lanuginosus* and *Thermoascus aurantiacus* grown on lignocelluloses", page 298 - page 302, see abstract page 298 and figure 3 page 301	15-35
A	--	1-14
X	Dialog Information Services, file 5, BIOSIS PREVIEWS, Dialog accession no. 4460997, BIOSIS no. 78034820, Kitpreechavanich V et al: "Production of xylan degrading enzymes by thermophilic fungi aspergillus- fumigatus and humicola-lanuginosa"; & J FERMENT TECHNOL 62 (1) 1984 63-70	15-35
A	--	1-14
X	WO 9217573 A1 (NOVO NORDISK A/S), 15 October 1992 (15.10.92), see abstract	1-2,15-16
A	--	3-14,17-35

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00046

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Dialog Information Services, file 5, BIOSIS PREVIEWS, Dialog accession no. 7137982, BIOSIS no. 88060727, Anand L et al: "Purification and properties of beta glucosidase from thermophilic fungus humicola-lanuginosa griffon and maublanc bunce"; & J FERMENT BIOENG 67 (6) 1989 380-386	15-17
A	--	1-14,18-35
X	CANADIAN JOURNAL OF MICROBIOLOGY, Volume 35, No 9, 1989, Ramesh K. Ganju et al, "Purification and characterization of two xylanases from chaetomium thermophile var. coprophile", page 836 - page 842, abstract	15
A	--	1-14,16-35
A	BIOTECHNOLOGY, Volume 10, November 1992, Anneli Törrönen et al, "The two major xylanases from trichoderma reesei: characterization of both enzymes and genes", page 1461 - page 1465, whole document	1-35
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A	MOLECULAR PLANT MICROBE INTERACTIONS, Volume 6, No 4, 1993, Patricia C. Apel et al, "Cloning and Targeted Gene Disruption of XYL1, a beta1, 4-Xylanase Gene from the Maize Pathogen Cochliobolus carbonum", page 467 - page 472, whole document	1-35
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A	WO 9421785 A1 (NOVO NORDISK A/S), 29 Sept 1994 (29.09.94), whole document	1-35
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A	WO 9324621 A1 (OY ALKO AB), 9 December 1993 (09.12.93), whole document	1-35
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 96/00046

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 7, 21 and 26
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see extra sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

The wording "..immunologically reactive with an antibody raised against.." does not define a property that is relevant in the context of the invention, as there is no direct link between the enzymatic activity and the immunological features (except for some unknown epitopes of the active site). Due to this vague definition claims 7,21 and 26 do not fulfil the requirements of PCT article 6 regarding clarity and conciseness.

The word "..homologous.." in claim 7, 21 and 26 is not considered to be clear and concise since it has not been specified to what extent the sequence is homologous with the DNA sequence/polypeptide corresponding to SEQ ID No. 1 (cf. PCT article 6). Furthermore, the definition of the DNA construct and corresponding polypeptide of the homologues of SEQ ID No. 1 referred to in claim 7, 21 and 26 must include those parts that encode the alleged inventive features of the xylanase.

The wording "..hybridize with the same oligonucleotide probe.." of claim 7, 21 and 26 is not considered to be clear and concise since the part, to which the oligonucleotide hybridizes with the analogue, is not restricted to include the part that encodes the alleged inventive features of the xylanase.(cf. PCT article 6)

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/04/96

International application No.
PCT/DK 96/00046

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9104673	18/04/91	DE-D,T- 69007115	09/06/94
		EP-A,A,B 0494916	22/07/92
		JP-T- 5500807	18/02/93
WO-A1- 9217573	15/10/92	CA-A- 2106484	03/10/92
		EP-A,A- 0507723	07/10/92
		EP-A- 0579672	26/01/94
		JP-T- 6506348	21/07/94
WO-A1- 9421785	29/09/94	NONE	
WO-A1- 9324621	09/12/93	NONE	

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